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Interactions between the *nod*$^+$ Kinesin-like Gene and Extracentromeric Sequences Are Required for Transmission of a Drosophila Minichromosome

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Summary

In this study, we demonstrate a role for extracentromeric sequences in chromosome inheritance. Genetic analyses indicate that transmission of the Drosophila minichromosome *Dp1187* is sensitive to the dosage of *nod*$^+$, a kinesin-like gene required for the meiotic transmission of achiasmate chromosomes. Minichromosome deletions displayed increased loss rates in females heterozygous for a loss-of-function allele of *nod* (*nod*/+). We have analyzed the structures of *nod*-sensitive deletions and conclude that multiple regions of *Dp1187* interact genetically with *nod*$^+$ to promote normal chromosome transmission. Most *nod*$^+$ interactions are observed with regions that are not essential for centromere function. We propose that normal chromosome transmission requires forces generated outside the kinetochore, perhaps to maintain tension on kinetochore microtubules and stabilize the attachment of achiasmate chromosomes to the metaphase spindle.

Introduction

Chromosome transmission and segregation during mitosis and meiosis require interactions between chromosomal DNA and the cellular machinery, including spindle microtubules and motor proteins. Cytogenetic studies have shown that the centromere of each chromosome plays a key role in these interactions, serving as the nucleation site for a complex (the kinetochore) that binds microtubules and facilitates movement (reviewed by Brinkley, 1991). Studies utilizing Saccharomyces cerevisiae and Schizosaccharomyces pombe have identified DNA elements that provide centromere activity during mitosis and meiosis (reviewed by Hegemann and Fleig, 1993; see also Baum et al., 1994). Similarly, our studies in Drosophila melanogaster have localized the essential core of the centromere to a 220 kb region (T. D. M. and G. H. K., submitted). However, some mitotic and meiotic activities of endogenous chromosomes are not encoded by the minimal regions necessary for chromosome transmission (the minimal centromere). The stability of artificial chromosomes in S. cerevisiae (Sleister et al., 1992) and S. pombe (Clarke and Baum, 1990) increases with overall size. Normal meiotic segregation in S. pombe requires a single copy central core plus adjacent repeated sequences (Clarke and Baum, 1990; Baum et al., 1994). Chromosome transmission in Drosophila requires at least 200 kb of repetitive sequences in addition to the essential core (T. D. M. and G. H. K., submitted). These observations raise a number of questions. What are the roles of extracentromeric regions in chromosome transmission? What are the *trans*-acting factors that interact with these regions?

Heterochromatin is a poorly understood component of the genome, but is known to contain repetitive sequences and to induce position-effect variegation (silencing) of nearby euchromatic genes (reviewed by John, 1988; Karpen, 1994). In multicellular eukaryotes, specific chromosome inheritance functions have been attributed to heterochromatic regions outside of the centromere. Studies in the mouse have correlated sites of sister chromatid cohesion with the highly repetitive satellite sequences found in heterochromatin (Lica et al., 1986). Disjunction of homologous chromosomes in Drosophila meiosis is also mediated by heterochromatin (McKee and Karpen, 1990; Hawley et al., 1993; M. H. Le and G. H. K., unpublished data). Additional roles for extracentromeric regions (heterochromatic and euchromatic) in chromosome inheritance remain to be determined.

How can we study the roles of extracentromeric regions in chromosome inheritance? Many structural and functional properties of metazoan chromosomes have been ascertained using the Drosophila minichromosome *Dp(1,f)1187* (*Dp1187*; Figure 1) (Karpen and Spradling, 1990, 1992; Tower et al., 1993; Zhang and Spradling, 1993; Thompson et al., 1994). This minichromosome contains all of the DNA elements necessary to promote efficient transmission and segregation in mitosis and meiosis. Furthermore, its small size (1300 kb) makes it amenable to molecular analysis. We have recently generated a large number of deletion derivatives of *Dp1187* and used them to characterize structural and functional characteristics of this minichromosome. The 1 Mb of centric heterochromatin in *Dp1187* contains three islands of complex DNA (single copy or middle repetitive DNA), called *Tahiti*, *Moorea*, and *Bora Bora*, interspersed with blocks of highly repetitive satellite DNA (Le et al., 1995). Analyses of the transmission behavior of *Dp1187* deletion derivatives demonstrated that *Bora Bora* contains the essential core of the centromere. However, 200 kb of satellite sequences flanking *Bora Bora* also are required for completely normal transmission (T. D. M. and G. H. K., submitted).

How can we identify the chromosomal regions that interact with specific gene products needed for inheritance? The molecularly defined deletion derivatives of *Dp1187* provide the means to manipulate specific chromosomal regions in vivo. Normally, deleting extracentromeric regions or reducing the dosage of inheritance genes (as in a mutation/+ heterozygote) does not affect inheritance. However, we hypothesized that severely deleted chromosomes may be especially sensitive to reduced dosage of a *trans*-acting gene. Deleting a *cis*-acting chromosomal region that is involved in the function of the *trans*-acting gene could increase dosage sensitivity, indicating a ge-

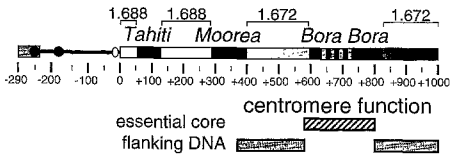


Figure 1. Structural and Functional Elements in *Dp 8-23*

Dp 8-23 is 1320 kb in size. The 1000 kb of centric heterochromatin (block with stippled gradient) originates from the basal portion of the X, while the subtelomeric heterochromatin (small stippled block) and euchromatin (black line), including the body color gene y^+ (open oval), originates from the distal tip of the X (Karpen and Spradling, 1990). The two stippled circles each represent a 14.5 kb ry^+ P element insertion (Karpen and Spradling, 1992; Tower et al., 1993). References to specific regions of *Dp 8-23* ignore the size increase due to the P elements, consequently the left tip of the chromosome is −290, the terminus of the parental *Dp1187* (Karpen and Spradling, 1992). The centric heterochromatin contains three islands of complex DNA (single copy or middle repetitive DNA), termed *Tahiti*, *Moorea*, and *Bora Bora*, which alternate with blocks that contain highly repetitive satellite DNA (1.688 and 1.672) (Le et al., 1995). A 220 kb region encompassing *Bora Bora* is essential for chromosome transmission and is proposed to encode kinetochore formation; however, completely normal chromosome transmission also requires 200 kb of flanking DNA on either side of *Bora Bora* (T. D. M. and G. H. K., submitted).

netic interaction. If true, we could characterize the interacting regions present throughout *Dp1187* by assaying the sensitivity of different deleted minichromosomes to gene dosage. Furthermore, extensively deleted minichromosomes could be used in genetic screens to identify novel inheritance genes.

We have tested this approach by characterizing the functional interactions of the *no distributive disjunction* (*nod*) gene with *Dp1187*. Complete loss of nod^+ function results in high levels of nondisjunction and loss of nonrecombinant (achiasmate) chromosomes during female meiosis I (Carpenter, 1973; Zhang and Hawley, 1990). The nod^+ gene encodes a kinesin-like putative microtubule-based motor (Zhang et al., 1990). Cytological studies suggest that nod^+ provides a mechanical force that, in the absence of recombination chiasmata, is necessary to maintain chromosome positioning during metaphase (Theurkauf and Hawley, 1992). Genetic studies have revealed that nod^+ function can be sensitized to reduced dosage (Knowles and Hawley, 1991), making it an ideal candidate for testing minichromosome sensitivity to gene dosage.

In this study, we demonstrate that *Dp1187* is sensitive to the dosage of nod^+. Deletions within *Dp1187* increased *nod* sensitivity, demonstrating that multiple regions of *Dp1187* interact functionally with nod^+. Genetic interactions occurred with most regions of *Dp1187*, including regions that are not required for centromere function. We propose that antipoleward forces generated on extracentromeric sequences stabilize chromosome transmission by maintaining tension on kinetochore microtubules. This approach can be used to localize chromosomal interacting regions for other inheritance genes and to identify novel inheritance genes.

Results

nod^+ Is Required for Transmission of *Dp1187*

Dp1187 is marked with the body color gene *yellow*$^+$ (y^+). The derivative *Dp 8-23* is identical to *Dp1187* except for the insertion of two P elements containing *rosy*$^+$ (ry^+) eye color genes into the subtelomeric heterochromatin (Tower et al., 1993). A simple genetic assay shows that *Dp 8-23* is efficiently transmitted during mitosis and meiosis. A *y; ry; Dp 8-23* (y^+ ry^+) female containing a single (monosomic) minichromosome per diploid genome is crossed to $X\wedge Y$, *y/O; ry* males. Approximately half of the progeny (52% transmission; Table 1) express y^+ and ry^+, indicating that the *Dp 8-23* monosome is inherited normally.

Efficient transmission of *Dp1187* and *Dp 8-23* requires nod^+ activity. Transmission of *Dp1187* from females homozygous for a *nod* mutation (nod^{b17}/nod^{b17}) was dramatically reduced from 52% to 3% (Table 1). Transmission of *Dp 8-23* from heterozygous *nod* females ($nod^{b17}/+$) was reproducibly reduced to 47%, slightly lower than the 52% transmission observed in nod^+ female siblings (differences of 5% or greater are statistically significant at a t value of 0.05 unless otherwise noted; see Experimental Procedures for statistical tests). The normal transmission from nod^+ sibling females demonstrates that the reduced transmission in heterozygotes is linked to the nod^{b17} chromosome and is not due to a dominant modifier of transmission present on another chromosome.

Other *nod* alleles have similar effects on *Dp 8-23* transmission (Table 1). Severe *nod* alleles reduced *Dp 8-23* transmission more than weaker alleles ($nod^{DTW} > nod^{DR3} \geqslant nod^{b17}$) (Wright, 1974; Rasooly et al., 1991; Zhang and Hawley, 1990). Heterozygotes for the hypomorphic nod^a allele (Carpenter, 1973) did not display reduced *Dp 8-23* transmission. However, nod^{b17}/nod^a reduced *Dp 8-23* transmission to 9% compared with 47% transmission for $nod^{b17}/+$ alone, demonstrating that nod^a can affect minichromosome transmission. Therefore, four alleles of *nod* behave similarly, which confirms that mutations at the *nod* locus are responsible for the reduced transmission of *Dp 8-23*, rather than a dominant modifier at another locus on the X chromosome.

nod Sensitivity Increases Owing to Chromosomal Deletions

Chromosomal deletions of *Dp1187* displayed increased sensitivity to nod^+ dosage. Transmission of the terminal deletion derivative *704* and the centric heterochromatin deletion derivative *γ1230* was comparable to *Dp 8-23* in nod^+ females (Table 1). However, partial loss of nod^+ function ($nod^{b17}/+$) reduced *704* transmission to 29%, dramatically lower than the 47% transmission of *Dp 8-23*. Similarly, transmission of *γ1230* was reduced to 24% in $nod^{b17}/+$ females. The differential transmission of *Dp1187* derivatives also was seen with other alleles of *nod*, and the magnitude of the effects was consistent with the severity of the allele (Table 1).

The *nod* sensitivity of deletion derivatives suggests nod^+ functions through sequences in the deleted regions. Telo-

Table 1. Effect of *nod* on Transmission of *Dp1187* Derivatives

Derivative	Deletion	+/+ (% ± σ)	(n)	*FM7*/+ (% ± σ)	(n)	nod^{b17}/nod^{b17} (%)	nod^{b17}/+ (% ± σ)	(n)	nod^{DTW}/+ (%)	nod^{DR3}/+ (% ± σ)	(n)	nod^{a}/+ (% ± σ)	(n)	nod^{b17}/nod^{a} (%)
Dp 8-23	–	52 ± 5	(65)	54 ± 5	(41)[a]	3[b,c]	47 ± 6	(70)	15	43 ± 11	(16)	56 ± 5	(15)	9[b]
704	Subtelomeric	55 ± 4	(9)	52 ± 7	(33)	ND[d]	29 ± 9	(22)	7	33 ± 10	(26)	53 ± 6	(13)	ND
γ1230	Centric Heterochromatin	51 ± 5	(32)	51 ± 7	(30)	ND	24 ± 10	(32)	8	25 ± 11	(24)	47 ± 8	(10)	ND

Monosome transmission from individual females of the specified genotypes was measured as described in Experimental Procedures, and the average percent transmission ± SD (σ) is reported here; n indicates the number of individual females assayed. Females were pooled in *nod/nod* and nod^{DTW}/+ crosses because of the reduced viability of progeny. Consequently, the variance could not be measured in these crosses.

[a] *FM7*/+ values are a combination of all siblings assayed for nod^{DTW}/+, nod^{DR3}/+, and nod^{a}/+. Statistical comparisons were made only between appropriate siblings.

[b] nod^{b17}/nod^{b17} generated 78% chromosome 4 nondisjunctional progeny. nod^{b17}/nod^{a} generated 77% nullo-4 progeny.

[c] Performed with *Dp1187*, which is identical to *Dp8-23* (Figure 1) except that it lacks the two *ry*$^+$ P insertions.

[d] ND, not done.

meric (*704*) and centric heterochromatin (*γ1230*) deletions did not reduce transmission in a *nod*$^+$ background. Partial loss of *nod*$^+$ function only slightly reduced the transmission of *Dp 8-23*. However, the partial loss of *nod*$^+$ function in combination with deletion of telomeric or centric heterochromatin resulted in substantial minichromosome instability. The deleted regions (*nod*$^+$ interacting regions) may contain one or several individual interaction sites for *nod*$^+$, or *nod*$^+$ may interact with the entire chromosome in a size-dependent manner. Genetic interactions could be direct, such as binding of DNA in these regions by nod protein, or indirect, mediated through other factors such as microtubules or chromosomal proteins.

nod$^+$ Interacts with Multiple Subtelomeric Regions

We assayed the *nod* sensitivity of a large number of terminal (e.g., *704*) and interstitial (e.g., *γ1111*) deletion derivatives to more precisely localize the chromosomal regions that interact with *nod*$^+$ (Figure 2A). Deletion of up to 50 kb of sequences from the subtelomeric heterochromatin (*3601* and *8-61A*) did not increase *nod* sensitivity relative to *Dp 8-23* (*3601* = 47%; *8-61A* = 49%). However, deletion of internal euchromatic regions in addition to the subtelomeric heterochromatin (*8-61B*, *γ878*, and *704*) substantially increased *nod* sensitivity (*8-61B* = 34%; *γ878* = 29%; *704* = 29%). Surprisingly, deletion of the euchromatin without deleting the subtelomeric heterochromatin (*γ1111*) did not increase *nod* sensitivity in comparison to *Dp 8-23* (45% transmission). All of these minichromosomes were transmitted normally from *nod*$^+$ female siblings, indicating that partial loss of *nod*$^+$ function is responsible for their instability.

We conclude that *nod*$^+$ interacts with three subtelomeric regions of *Dp1187* (summarized at the bottom of Figure 2A). The *nod* sensitivity of *3601* and *8-61A* is comparable to *Dp 8-23*, demonstrating that terminal deletions per se are transmitted normally in a *nod*/+ background and suggesting that the −290 to −240 kb region is not essential for *nod*$^+$-mediated transmission. The increased *nod* sensitivity of *8-61B*, *γ878*, and *704* demonstrates that two functionally separable interacting regions are present in −240 to −120 and −120 to −80 (Figure 2A). However, removal of these two regions in *γ1111* did not increase *nod* sensitivity. This suggests that the −290 to −240 region of subtelomeric heterochromatin also interacts with *nod*$^+$ and can fully compensate for loss of the two internal regions. Reciprocally, the presence of the two internal regions can compensate for loss of the −290 to −240 region, as in *8-61A* (summarized at the bottom of Figure 2A). The subtelomeric regions of *Dp1187* originated from the distal tip of the X, approximately 40 Mb away from the X centromere (Karpen and Spradling, 1990). Therefore, *nod*$^+$ genetically interacts with regions located outside of the centromere.

nod$^+$ Interacts with Multiple Regions of the Centric Heterochromatin

γ-Mutagenesis of *Dp 8-23* (Le et al., 1995) generated three internal deletions, removing portions of the centric heterochromatin as shown in Figure 2B. These derivatives are

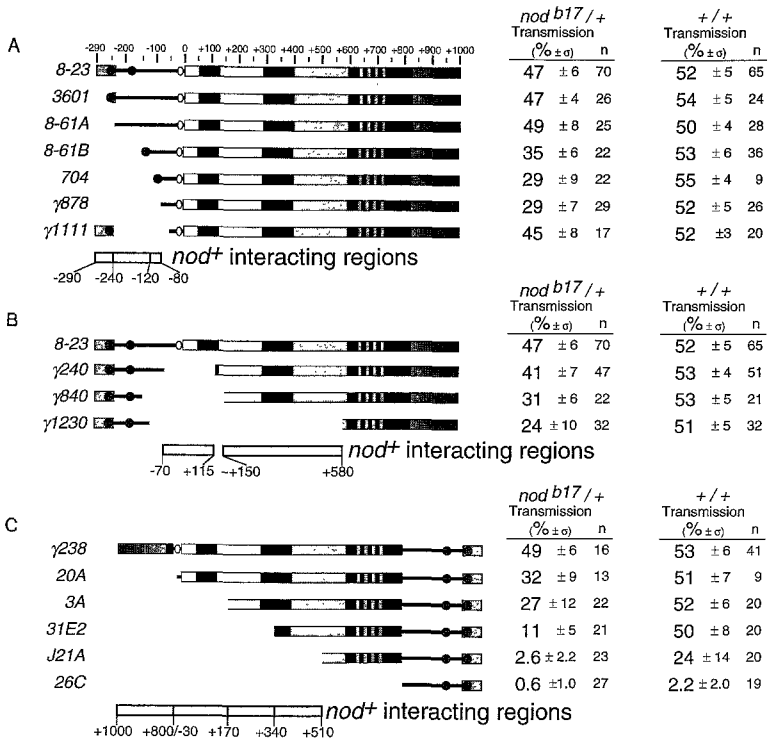


Figure 2. Mapping *nod*$^+$ Interacting Regions within *Dp1187*

(A–B) Terminal and interstitial deletions of *Dp1187* were generated by P element mutagenesis or γ-irradiation and their structures determined by pulsed-field Southern blot analysis (Karpen and Spradling, 1992; Tower et al., 1993; Zhang and Spradling, 1993; Le et al., 1995). The transmission of these *Dp1187* derivatives was measured in *nod*$^+$ (+/+) and *nod*/+ sibling females and reported as percent transmission ± SD (see Experimental Procedures for the crosses and an explanation of the observed variation); n indicates the number of individual females assayed. All derivatives are stable in a *nod*$^+$ background (~50% transmission; T. D. M. and G. H. K., submitted). The *nod*$^+$ interacting regions (stippled blocks) indicated below are the minimum number of interactions suggested by the data and may represent one or many interactions occurring within each region.

(C) *γ238* was generated from *Dp 8-23* by the inversion of sequences between −30 and +800, moving the *ry*$^+$ marker genes to the right end of the chromosome (Le et al., 1995). Terminal deletions of *γ238* were subsequently generated by γ-irradiation (T. D. M. and G. H. K., submitted), and their *nod* sensitivity was measured as in (A)–(B). Only *J21A* and *26C* are unstable in a *nod*$^+$ background. All other symbols are as described in Figure 1.

transmitted normally in a *nod*$^+$ background (T. D. M. and G. H. K., submitted; also see Figure 2B, +/+), but were unstable in *nod*/+ heterozygotes. *γ240*, which deletes sequences from −70 to +115, was transmitted to only 41% of progeny in a *nod*/+ background. More dramatically, the 300 kb deletion in *γ840* reduced transmission to 31%, and the 700 kb deletion in *γ1230* decreased transmission to 24%.

The transmission behavior of these three derivatives suggests that *nod*$^+$ interacts with at least part of the centric heterochromatin. The increased *nod* sensitivity of *γ240* maps a *nod*$^+$ interacting region to −70 to +115, which contains both euchromatin and heterochromatin. Portions of the two internal subtelomeric *nod*$^+$ interacting regions are deleted in *γ840*, making it difficult to determine which regions are responsible for the increased *nod* sensitivity of *γ840*. However, the +150 to +580 region of centric heterochromatin removed in *γ1230* must interact with *nod*$^+$, since *γ1230* removes less euchromatin than *γ840* yet is more sensitive to partial loss of *nod*$^+$ function.

Interactions between *nod*$^+$ and the centric heterochromatin were characterized in detail with a series of derivatives of the *γ238* inversion (Figure 2C). This rearrangement of *Dp 8-23* (Le et al., 1995) positions the *y*$^+$ and *ry*$^+$ markers on opposite sides of the centric heterochromatin. *γ238* was used to generate a large number of terminal deletions that lack various portions of the centric heterochromatin (T. D. M. and G. H. K., submitted). The majority of these terminal deletions are transmitted normally in a *nod*$^+$ background.

The behavior of the *γ238* terminal deletions demonstrates that *nod*$^+$ interacts with multiple regions of the centric heterochromatin. The *γ238* inversion did not increase *nod* sensitivity in comparison to *Dp 8-23*. However, deletions of the centric heterochromatin substantially increased sensitivity to *nod*$^+$ dosage. A terminal deletion that removed the +800 to +1000 region (*20A*; Figure 2C) reduced *nod*/+ transmission to 32%, 17% lower transmission than *γ238*. The stability of the terminal deletions *3601* and *8-61A* demonstrates that introduction of a terminal deletion per se does not cause *nod* sensitivity. Thus, the increased *nod* sensitivity of *20A* is likely due to the interaction of *nod*$^+$ with +800 to +1000. Removal of additional sequences from −25 to +170 (*3A*) decreased *nod*/+ transmission only slightly, to 27% (compared with *20A* = 32%; p = 0.13). Further deletion of sequences from +170 to +340 (*31E2*) substantially lowered *nod*/+ transmission to 11%. The decreased transmission of *20A*, *3A*, and *31E2* supports the conclusion that three *nod*$^+$ interacting regions exist at +800 to +1000, −25 to +170, and +170 to +340 (bottom of Figure 2C).

nod$^+$ Interacts with Regions Adjacent to *Bora Bora*

Our previous studies have shown that completely normal chromosome function requires *Bora Bora* (the essential core; see Figure 1) plus flanking DNA located at +800 to +1000 or +370 to +600 (T. D. M. and G. H. K., submitted). *nod*$^+$ interacts strongly with the +800 to +1000 region (see preceding section). However, the instability of *J21A* and *26C* in *nod*$^+$ females (Figure 2C) hinders unequivocal con-

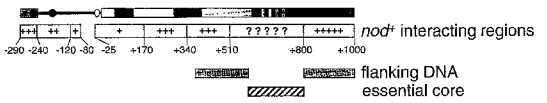


Figure 3. The Complete Map of *nod*+ Interacting Regions

nod+ interacting regions mapped in Figures 2A and 2C are compared with the regions required for centromere function. The number of pluses indicates the relative strength of different *nod*+ interacting regions, as described in the Results. Interacting regions mapped in Figure 2B are not included because the *γ238* derivatives used in Figure 2C provide better resolution through the centric heterochromatin. *nod*+ interactions with the essential core of the centromere (hatched block) could not be ascertained (question marks); however, the flanking DNA (stippled blocks) required for completely normal transmission in *nod*+ females correlates well with *nod*+ interacting regions.

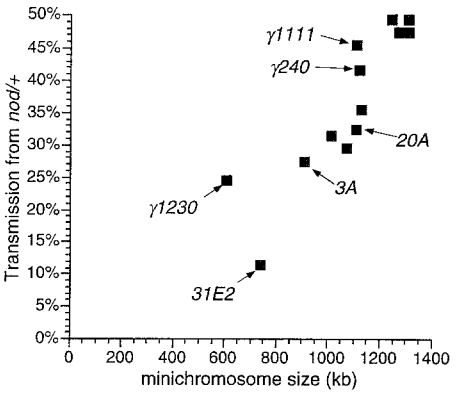


Figure 4. Chromosome Size Is Not an Absolute Predictor of Stability in *nod*/+ Females

The transmission of *Dp1187* derivatives from *nod*^{b17}/+ females is plotted as a function of minichromosome size. The positive correlation suggests that most regions of *Dp1187* act additively in their interactions with *nod*+. However, the correlation is not strictly linear. Specific derivatives described in the Results and indicated on the graph suggest that *nod*+ interacting regions differ in overall strength. *J21A* and *26C* are not included because of their instability in a *nod*+ background.

clusions about interactions with the flanking DNA in the +370 to +600 region and with *Bora Bora*.

The *J21A* derivative removes all but 90 kb of the centric heterochromatin flanking *Bora Bora* (Figure 2C). This derivative is more sensitive to *nod*+ dosage than is *31E2* (transmission of *J21A* in *nod*/+ is one ninth that seen in *nod*+, compared with one fifth for *31E2*), suggesting an interaction of *nod*+ with the +340 to +510 region. Given the clear interaction of *nod*+ with the +800 to +1000 region (see above), it seems likely that both regions of flanking DNA that stabilize transmission in *nod*+ females interact with *nod*+ (Figure 3).

26C is acentric (T. D. M. and G. H. K., submitted). Since such chromosomes are highly unstable (2% transmission in *nod*+ females; see Figure 2C), an assessment of *nod*+ interaction with *Bora Bora* is difficult (indicated by question marks in Figure 3). However, we do see decreased transmission of *26C* in *nod*/+ females compared with *nod*+, consistent with an interaction of *nod*+ with this acentric fragment.

nod+ Interacting Regions Vary in Strength

Theurkauf and Hawley (1992) proposed that the antipoleward force provided by *nod*+ is proportional to chromosome size and acts along the chromosome arms. To test this hypothesis, we examined the relationship between chromosome size and stability in *nod*/+ females (Figure 4). In general, larger derivatives transmit better from *nod*/+ females than smaller derivatives. The positive correlation between chromosome size and stability in *nod* heterozygotes suggests that most regions of *Dp1187* interact genetically with *nod*+.

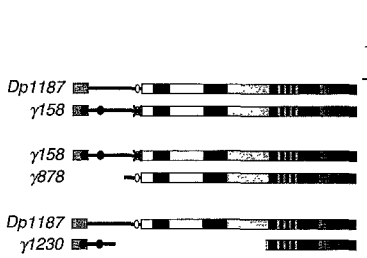
However, the size of *Dp1187* derivatives is not an absolute predictor of their stability in *nod*/+ females. *γ1111*, *γ240*, and *20A* are all similar in size (~1110 kb), but transmitted differently from *nod*/+ females (*γ1111* = 45%; *γ240* = 41%; *20A* = 32%; Figure 4). *31E2* is 21% larger than *γ1230* but was more sensitive to *nod*+ dosage. And *γ1230* and *3A* transmitted at approximately the same level from *nod*/+ (24% and 27%, respectively) despite their 300 kb difference in size.

Exceptions to the linear relationship between size and stability suggest that regions of the minichromosome differ in the magnitude of their genetic interaction with *nod*+. If the strength of regions are additive, then the relative strength of different regions can be determined by comparing regions present in chromosomes with similar *nod* sensitivity (see Figure 3; pluses indicate relative interaction strength). For example, *γ1230* and *3A* transmitted similarly in *nod*/+ females, suggesting that the +800 to +1000 region present in *γ1230* (200 kb, +++++) interacts with *nod*+ as strongly as the combined +170 to +340 and +340 to +510 regions present in *3A* (340 kb total, each given +++). Similarly, *γ1111* and *8-61A* showed comparable *nod* sensitivity, suggesting that the 50 kb region of subtelomeric heterochromatin (−290 to −240, +++) shows *nod*+ interaction comparable to a 160 kb region of euchromatin (−240 to −120, ++; −120 to −80, +).

Overall, *nod*+ interacts with most regions of *Dp1187* (see Figure 3), suggesting that *nod*+ interacting regions will be found throughout other chromosomes. However, different regions of *Dp1187* vary in their ability to interact with *nod*+. These variations could represent either differences in the number of individual interaction sites within a region or the availability of that region to interact with *nod*+.

Partial Loss of *nod*+ Function Substantially Increases Nondisjunction of *Dp1187*

We have used the transmission of minichromosomes without pairing partners (monosomes) to characterize *nod*+ interactions with the chromosome. However, naturally occurring Drosophila chromosomes usually have a pairing partner during meiosis I. Chromosomes lacking recombination chiasmata (achiasmate chromosomes) require *nod*+ both for transmission and for faithful segregation from their pairing partner. Loss of *nod*+ function increases nondisjunction and loss of the fourth chromosomes, which are always achiasmate (Carpenter, 1973; Zhang and Hawley, 1990). An achiasmate X chromosome pair (such as X/*FM7*) nondisjoins only ~1% of the time in *nod*+ and



	nod b17/+ Transmission (%±σ)	nod b17/+ True Nondisjunction (%±σ)	nod b17/+ n	+/+ Transmission (%±σ)	+/+ True Nondisjunction (%±σ)	+/+ n
Dp1187 / γ158	42±8 45±8	36±12	15	49±5 53±5	8±3	18
γ158 / γ878	51±6 29±7	32 ±6	23	52±4 50±6	8±7	14
Dp1187 / γ1230	48±8 25±9	29±11	17	47±6 53±7	10±5	14

Figure 5. *nod* Increases Nondisjunction of *Dp1187* Derivatives

Rates of chromosome transmission and nondisjunction were measured in *nod*$^+$ (+/+) and *nod*^{b17}/+ females. The presence of a pairing partner did not alter the observed chromosome transmission rates (compare with Figures 2A and 2B). Partial loss of *nod*$^+$ function (*nod*/+) dramatically increased the frequency of nondisjunction compared with *nod*$^+$ female siblings (+/+). True nondisjunction (TND) rates reported are corrected for chromosome loss rates, as described in Experimental Procedures. *γ158* is identical to *Dp 8-23* except for a point mutation in the *y*$^+$ gene, resulting in a *y*$^-$ *ry*$^+$ phenotype (Le et al., 1995; K. Donaldson and G. H. K., unpublished data).

nod/+ females, but this increases to 51% in *nod*$^+$ females (Zhang and Hawley, 1990). These observations raise two questions. Are *nod*$^+$ interacting regions required when a pairing partner is present? Does *nod*-induced nondisjunction depend on the same interacting regions? To answer these questions, we measured the rate of minichromosome nondisjunction and loss in the presence of a minichromosome pairing partner.

We used differentially marked minichromosomes (e.g., *y*$^-$ *ry*$^+$ and *y*$^+$ *ry*$^-$) to measure the transmission of each minichromosome independently when a pairing partner was present. The presence of a pairing partner does not influence chromosome loss rates caused by partial loss of *nod*$^+$ function. In *nod*/+ females, *Dp1187* and *γ158* transmitted at 42%–51% in the presence of a homologous pairing partner (Figure 5), comparable to the monosome transmission of *Dp 8-23* (47%; see Figure 2A) and *γ158* (44%; data not shown). The *nod*-sensitive derivative *γ878* transmitted at 29% regardless of the presence or absence of *γ158* (compare with Figure 2A). *γ1230* transmitted at 25% in the presence of *Dp1187*, comparable to the 24% level reported for monosomes. The similar *nod* sensitivity of *γ878* and *γ1230* with and without a pairing partner indicates that the presence of a pairing partner does not influence the activity of specific *nod*$^+$ interacting regions.

Homologous *Dp1187* derivatives were able to pair and disjoin during meiosis I in *nod*$^+$ females (Figure 5; 8%–10% true nondisjunction [TND] rates, corrected for chromosome loss; see Experimental Procedures). Partial loss of *nod*$^+$ function dramatically increased the frequency of nondisjunction. The nondisjunction of *Dp1187* and *γ158* increased from 8% TND in *nod*$^+$ females to 36% TND in *nod*/+ females. Similarly, *γ158/γ878* nondisjunction increased from 8% TND to 32% TND, and *Dp1187/γ1230* nondisjunction increased from 10% TND to 29% TND. The dramatic increases in nondisjunction do not appear to depend on specific *nod*$^+$ interacting regions mapped within *Dp1187*, since all three pairs of chromosomes showed similar increases in nondisjunction.

Discussion

We have shown that the transmission and segregation of *Dp1187* and its derivatives are sensitive to partial loss of *nod*$^+$ function. Deletion of specific regions of the subtelomeric and centric heterochromatin caused instability in *nod*/+ females, demonstrating that *nod*$^+$ interacts genetically with these regions. We conclude that multiple extracentromeric regions of *Dp1187* functionally interact with *nod*$^+$ to promote chromosome transmission and segregation.

nod$^+$ Interacting Regions Are Distributed Throughout *Dp1187*

Smaller chromosomes are more sensitive than larger chromosomes to loss of *nod*$^+$ function. Complete loss of *nod*$^+$ function results in low levels of X chromosome loss but high levels of fourth chromosome and *Dp1187* loss (Carpenter, 1973; Zhang and Hawley, 1990; this study; transmission of X = 48%, fourth chromosome = 14%, *Dp1187* = 3%). Partial loss of *nod*$^+$ function produces a less severe gradient of *nod* sensitivity: X and fourth chromosome transmissions are unaffected, but *Dp1187* transmission is reduced. Removing specific regions of *Dp1187* further increased sensitivity to partial loss of *nod*$^+$ function, serving as the basis for our mapping of *nod*$^+$ interacting regions.

The wide distribution of *nod*$^+$ interacting regions within *Dp1187* (Figure 3) suggests that most regions of the genome interact with *nod*$^+$. However, some *nod*$^+$ interacting regions within *Dp1187* are stronger than others. The strongest *nod*$^+$ interacting regions are found in heterochromatin (−290 to −240 and +800 to +1000; Figure 3), suggesting that heterochromatin plays a key role in *nod*$^+$-mediated chromosome transmission.

Most *nod*$^+$ interactions are observed in regions that are not essential for centromere function (Figure 3). It is surprising that this putative microtubule motor protein is interacting genetically with regions outside of the kinetochore. *nod*$^+$ also interacts with the regions flanking *Bora Bora* (+370 to +600 or +800 to +1000; Figure 3), which are necessary for completely normal transmission in *nod*$^+$ females. This correlation suggests that normal chromosome transmission in *nod*$^+$ females requires *nod*$^+$ interactions with sequences flanking *Bora Bora*. Without sufficient *nod*$^+$ interacting regions, minichromosomes may become unstable, even though two copies of the *nod*$^+$ gene are present.

The Nature of Genetic Interactions between *nod*$^+$ and the Chromosome

Why would specific regions of *Dp1187* be required for

transmission in *nod*/+ females and not *nod*$^+$ females? It is likely that the dosage of both *trans*-acting factors (e.g., *nod*$^+$) and *cis*-acting regions (*nod*$^+$ interacting regions) are important to normal inheritance. For *Dp1187*, deletion of a specific chromosomal region or partial loss of *nod*$^+$ function did not strongly affect transmission, but the combination resulted in high levels of instability. Studies on *nod* and another meiotic kinesin-like gene, *nonclaret disjunctional* (*ncd*) (Knowles and Hawley, 1991), suggest that dose sensitivity can be extended to include two *trans*-acting factors. Females heterozygous for *nod* or *ncd* display normal levels of X and fourth chromosome nondisjunction. But transheterozygous females (*nod*/+; *ncd*/+) show increased levels of fourth chromosome nondisjunction, indicating a genetic interaction between *nod*$^+$ and *ncd*$^+$.

The decreased chromosome transmission observed in our study could result from genetic interactions occurring in mitosis, meiosis, or both. However, several lines of evidence suggest that *nod*$^+$ interacting regions are required predominantly during meiosis I. Characterization of the loss-of-function *nod* phenotype indicates that most loss occurs during meiosis I (Carpenter, 1973; Zhang and Hawley, 1990). Loss of *nod*$^+$ function can also cause chromosome loss in early preblastoderm mitoses, generating mosaic progeny (e.g., half y^+ and half y^-). However, in our studies partial loss of *nod*$^+$ function rarely generated early mitotic loss events (data not shown), suggesting that most *nod*-induced loss occurred in the female parent and not in the progeny. Finally, the elevated frequencies of homolog nondisjunction observed in *nod*/+ females indicates part of the increased *nod* sensitivity of *Dp1187* derivatives must occur during meiosis I. It seems likely that the primary requirement for *nod*$^+$ interacting regions will be during meiosis I.

nod$^+$ Interacting Regions Are Likely to Be Sites of Direct Nod-DNA Binding

What is the underlying basis for the genetic interactions observed in our study? The *nod*$^+$ chromosome genetic interaction could be direct, such as binding of DNA by nod protein, or indirect, mediated through other factors such as microtubules or chromosomal proteins.

Recent biochemical and cytological studies suggest that the functional *nod*$^+$ interactions mapped in our study involve direct binding of DNA sequences by nod protein. Afshar et al. (1995 [this issue of *Cell*]) demonstrated that bacterially expressed nod protein binds DNA, including the 1.672 $(AATAT)_n$ satellite repeat but not the 1.705 $(AAGAGAG)_n$ satellite repeat. Furthermore, indirect immunofluorescence with affinity-purified nod antibodies showed that nod is localized to the chromosomes during female meiosis I. Our functional studies complement the biochemical and cytological observations of Afshar et al. (1995), demonstrating that *nod*$^+$ interactions with multiple extracentromeric regions are essential to transmission. Interestingly, one region of *Dp1187* with a particularly strong *nod*$^+$ interaction (+800 to +1000; Figure 3) contains a large block of the $(AATAT)_n$ satellite (Figure 1) (Le et al., 1995), correlating well with the in vitro binding activity of nod. *nod*$^+$ may interact with other regions because they contain AT-rich DNA (e.g., the 1.688 satellite; 70% AT; Hsieh and Brutlag, 1979).

We rely on the binding studies of Afshar et al. (1995) to propose a mechanism for the differential *nod* sensitivity of deleted minichromosomes. *nod*$^+$ oocytes are likely to have a high concentration of nod protein, leading to a high proportion of nod-binding sites being occupied. In *nod*$^+$ oocytes, a 620 kb chromosome (e.g., *y1230*) can bind enough nod to stabilize chromosome transmission (51% transmission observed). Reducing the dosage of *nod*$^+$ (*nod*/+) likely reduces the amount of nod protein, lowering the proportion of occupied sites. Under nod-limiting conditions, even a 1300 kb chromosome (e.g., *Dp 8-23*) cannot recruit enough nod for normal transmission, resulting in elevated loss rates (47% transmission). A 620 kb chromosome with fewer nod-binding sites (e.g., *y1230*) is even worse off: it can only recruit enough nod to allow moderate transmission (24% transmission).

A Model for *nod*$^+$ Function in Segregation and Transmission

Partial loss of *nod*$^+$ function leads to a dramatic increase in the nondisjunction of all the chromosome pairs studied. High rates of nondisjunction of two 1300 kb minichromosomes (*Dp1187* and *y158*, 36% TND; Figure 5) can occur without a substantial increase in chromosome loss (transmission of *Dp1187* = 42%; *y158* = 45%). Deletion of specific *nod*$^+$ interacting regions results in increased levels of chromosome loss, but does not affect the observed levels of nondisjunction. The X chromosome shows a similar disparity: homozygous *nod* females show high levels of X nondisjunction with low levels of chromosome loss (Zhang and Hawley, 1990; 51% TND, and 48% transmission of each X).

How might *nod* induce both chromosome nondisjunction and loss? Theurkauf and Hawley (1992) observed that achiasmate chromosomes are frequently detached from the meiosis I spindle in *nod*$^-$ females and suggested that detached chromosomes reattach to the spindle at a low frequency. They proposed that nondisjunction would occur when both homologs reattach to the same spindle pole and that loss occurs when reattachment fails. They suggested that the nod kinesin-like protein provides an antipoleward force (the so-called polar wind; reviewed by Carpenter, 1991; Rieder and Salmon, 1994; Fuller, 1995 [this issue of *Cell*]) that is required to hold achiasmate chromosomes on the metaphase spindle. Loss of *nod*$^+$ function was proposed to result in precocious segregation to the spindle pole and subsequent detachment from kinetochore microtubules. Afshar et al. (1995) have extended this model by demonstrating that nod protein is located on the chromosome arms.

The combined cytological, biochemical, and genetic data strongly support the idea that nod provides a force that counteracts poleward forces generated at the kinetochore (Afshar et al., 1995; Fuller, 1995). However, this model must be modified to explain why loss of *nod* function (*nod*/*nod* or *nod*/+) can induce high levels of X and *Dp1187* nondisjunction with only low levels of chromosome loss.

We propose that *nod*$^+$ provides forces that are neces-

sary both to maintain spindle attachment and to reattach to the spindle. Spindle detachment must result from loss of *nod*$^+$ function, since the X and fourth chromosomes detach in *nod*$^-$ oocytes (Theurkauf and Hawley, 1992). Spindle reattachment must also depend on *nod*$^+$, since *nod* homozygotes show high levels of loss of the fourth chromosome and *Dp1187*. Why is *nod*$^+$ function so central to maintaining these spindle attachments? Studies have shown that tension is required to maintain kinetochore attachments to microtubules (Nicklas and Ward, 1994) and that tension may be produced by antipoleward forces (Rieder and Salmon, 1994). We propose that the primary function of the *nod*$^+$ putative microtubule motor is to interact with nonkinetochore microtubules and extracentromeric DNA to produce a force that maintains tension on kinetochore microtubules. This tension is proposed to be necessary in female meiosis to maintain kinetochore attachment to the spindle and to promote kinetochore reattachment. Tension-producing forces mediated by *nod*$^+$ could involve either interchromosomal interactions or interactions with the near pole (antipoleward forces) or the opposite pole (contrapoleward forces) with respect to the kinetochore microtubules.

Both chromosome nondisjunction and loss can be accounted for with this model. Metaphase in meiosis I is quite long (King, 1970), and spindle attachment must be efficiently maintained for the duration. If *nod*$^+$ function is reduced or absent, achiasmate chromosomes will not be able to stabilize attachment to kinetochore microtubules, resulting in detachment while still near the metaphase plate. Small chromosomes may spend the majority of the time detached from the spindle, resulting in a high frequency of loss. Larger chromosomes may partially overcome the dependence on *nod*$^+$ function because of drag resulting from their physical size, providing some tension and reducing the probability of detachment. However, a single oscillation in attachment will allow chromosomes to reorient while still near the metaphase plate, effectively randomizing disjunction. Thus, this model proposes that chromosome transmission requires cooperative interactions between kinetochore and extracentromeric forces to maximize stability.

Exploring the Roles of Extracentromeric Sequences in Chromosome Inheritance

Extracentromeric sequences provide a number of functions important to chromosome inheritance. We have demonstrated that most of the extracentromeric sequences in *Dp1187* interact with *nod*$^+$ to stabilize chromosome transmission in Drosophila females. Recombination chiasmata can also ensure normal segregation (Carpenter, 1984). Interestingly, *nod*$^+$ is not essential to chromosome transmission in Drosophila male meiosis, even though all chromosomes are achiasmate. Perhaps spindle attachments and normal disjunction are ensured by interactions between extracentromeric regions and unidentified genes analogous to *nod*$^+$. Extracentromeric heterochromatin also is necessary for the disjunction of achiasmate homologous chromosomes in Drosophila females (Hawley et al., 1993; M. H. Le and G. H. K., unpublished data) and for the maintenance of sister chromatid adhesion in many species (Lica et al., 1986; Miyazaki and Orr-Weaver, 1994). Antipoleward forces are observed in mitosis and are thought to act all along the chromosome arms (Cassimeris et al., 1994; reviewed by Rieder and Salmon, 1994). The chromosomal kinesin-like protein Xklp1 may provide this mitotic antipoleward force in Xenopus (Vernos et al., 1995 [this issue of *Cell*]). It is possible that all of these functions act to balance forces applied at the kinetochore, maintaining tension on kinetochore microtubules and ensuring proper mitotic and meiotic segregation.

Many of the genes involved in the functions of extracentromeric sequences remain to be identified. The sensitivity of *Dp1187* and its derivatives will provide an important tool for elucidating the inheritance functions of extracentromeric and centromeric sequences. Previous screens for dosage-sensitive genetic interactions have been successful in identifying components of the Ras pathway (Simon et al., 1991) and *trans*-acting genes that interact with β-tubulin (Regan and Fuller, 1988). A similar approach utilizing the dosage sensitivity of deleted minichromosomes will serve as an efficient assay to screen for novel genes involved in chromosome inheritance and to identify the chromosomal regions necessary for their function.

Experimental Procedures

Stocks and Chromosomes

The *FM7a* balancer chromosome (referred to here as *FM7*), as well as *nod*^{a} and *FM7, nod*^{b17} (referred to here as *nod*^{b17} or just *nod*) are described by Zhang and Hawley (1990); *nod*^{DTW} and *nod*^{DR3} are described by Rasooly et al. (1991). *YSX·YL, In(1)EN, y* (referred to here as *X^Y*) and all marker genes are described by Lindsley and Zimm (1992). *Dp1187* and the derivatives are described elsewhere (Karpen and Spradling, 1990, 1992; Tower et al., 1993; Zhang and Spradling, 1993; Le et al., 1995; T. D. M. and G. H. K., submitted). All stocks were crossed into a *ry*^{506} background. The *nod* and *FM7* stocks used in this study were the gift of R. S. Hawley.

Genetics

The parental cross *nod/+; ry* ♀♀ × *y; ry; Dp* ♂♂ was used to produce *nod/+* or *+/+* female siblings that contain a single copy of the minichromosome. Any difference in the transmission from these sibling females can be attributed to the X chromosomes, since all other chromosomes are equally distributed to both types of females. Monosome transmission was determined by crossing single *nod/+; Dp* or *+/+; Dp* virgin females to three *X^Y, y/O; ry* males and then scoring their progeny. The *X^Y* chromosome was used to suppress variegation of the *y*$^+$ and *ry*$^+$ genes observed in some *Dp1187* derivatives. The cross produces both *X/X^Y* and *X/O* progeny, but *X/O* males were not scored owing to their enhanced variegation. The *nod*^{b17} allele is present on an *FM7* balancer chromosome marked with *y B w*; all other alleles required *FM7* in *trans* to *nod* to prevent X recombination, provide a copy of *nod*$^+$, and distinguish *nod/+* from *+/+* test animals. Parental crosses using *nod*^{DTW}/FM7 females produced some X nondisjunction events that could be identified as *y*$^+$ *B* progeny.

Dp transmission was calculated as the number of female progeny expressing the *Dp* marker phenotype (*y*$^+$ or *ry*$^+$) divided by the total number of female progeny and multiplied by 100%. A minimum of 30 progeny per female were scored for the data in Table 1 and of 50 progeny per female for all other data. Individual females produced an average of 72 scorable progeny for the *γ238* derivatives presented in Figure 2C and 129 scorable progeny for the data in Figures 2A, 2B, and 5. Assaying transmission from individual females allowed us to determine the variability between different germlines, as described below. The transmission and nondisjunction rates presented in Figures 2 and 5 were measured in at least two independent experiments. They reproduced to within 3% in almost all cases.

Dp1187 and fourth chromosome transmission from *nod*$^+$ females was measured with the cross *nod*^{b17}/*nod*^{b17}; *spa*^{pol}; *Dp1187* (y^+) ♀♀ × *y/Y; C(4)RM, ci ey*^{R}/O ♂♂, which allowed both nullo-4 and diplo-4 exceptions to be distinguished. The frequency of fourth chromosome nondisjunction was calculated as in Zhang and Hawley (1990). A similar cross was used to measure transmission rates from *nod*^{b17}/*nod*^{a} females, except the maternal fourth chromosome was not marked with *spa*^{pol}, so only nullo-4 exceptions could be distinguished.

For the nondisjunction analysis, crosses such as *nod*^{b17}/*y; ry; Dp1187, y*$^+$ ♀♀ × *y/Y; ry; γ158, ry*$^+$ ♂♂ were used to produce *nod*/+ and *nod*$^+$ female progeny carrying two minichromosomes (e.g., *Dp1187* and *γ158*). Single virgin female siblings were then crossed to *X^Y, y/O; ry* males, and the frequencies of y^+ ry^+, y^- ry^+, y^+ ry^-, and y^- ry^- progeny were measured. The overall frequencies of y^+ or ry^+ progeny reflect the transmission rates of each minichromosome. For each female assayed, the TND rate corrected for chromosome loss was calculated with the following formula: 50% × [frequency y^+ ry^+ / (frequency y^+ × frequency ry^+)]. The numerator is the observed frequency of progeny receiving both minichromosomes (unequivocal nondisjunction events); the denominator is the frequency expected if both minichromosomes segregated independently. This ratio will equal 1 if segregation occurs at random; it is multiplied by 50% to specify random disjunction as 50%. The average TND ± SD was then calculated for all the females assayed, as with the transmission rates.

Statistical Tests

The variation between germlines can be measured as the standard deviation σ observed for the transmission rates of independently measured females: $\sigma 2 = [n\Sigma x^2 - (\Sigma x)^2] / [n(n-1)]$, where n is the number of female germlines assayed and x is the transmission rate of each female. Average transmission was calculated as $\Sigma x/n$; i.e., each female is given equal weight regardless of the number of progeny produced. The distribution of transmission frequencies conforms to a normal distribution, except when the transmission rate is very low, as for the acentric derivative *26C*. Consequently, the Student's t test can accurately compare these distributions. Differences in transmission of 5% or more are significant at a t value of 0.05 unless otherwise noted. Averages, standard deviations, and p values were calculated using the T Test: Two-Sample Assuming Unequal Variances feature included in the Analysis Toolpak of Microsoft Excel 5.0 for the Macintosh.

The test results revealed that the variation observed between individual female germlines is higher than would be predicted by a binomial distribution. For example, 22 trials of *nod/+; 3A* gives 27% ± 12% transmission, but Monte Carlo simulations show this variation should fall in the range of 4.3%–6.7%. This suggests that individual meioses within and between germlines are not independent events: meioses occurring in the same germline could behave similarly because they have similar levels of *nod*$^+$ expression or germline clones have been produced owing to mitotic instability. It should be noted that the variation reported for the *γ238* derivatives (Figure 2C) is elevated because fewer progeny per female were scored.

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